United States Patent Office

2,955,110

Patented Oct. 4, 1960

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2,955,110

SUBSTITUTED PTERIDINES AND METHOD OF PREPARING THE SAME

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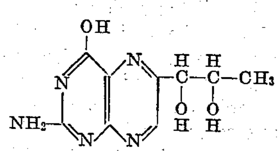
No Drawing. Filed Jan. 13, 1955, Ser. No. 481,704

4 Claims. (Cl. 260—251.5)

This invention relates to a new biologically active substance. More particularly, it relates to a biologically active substituted pteridine and a method for the preparation of the same.

In the past substituted pteridines such as pteroylglutamic acid, commonly called folic acid, have been found to be active in stimulating the growth of bacteria such as *Streptococcus fecalis* R. and to possess vitamin-like properties. Subsequently it was found that folic acid was also useful in stimulating hemaglobin formation and in the treatment of agranulocytosis.

We have now found a new substituted pteridine which is biologically active and is an essentially nutrient required for the growth of *Crithidia fasciculata*. This latter organism has been suggested as a test organism for the preliminary evaluation of antimalarials (Proceedings Society Experimental Biology & Medicine, 85, 117 (1954)). This test organism requires an exogenous source of folic acid (or closely related compounds) for growth, to which it responds quantitatively. The new growth factor for *Crithidia fasciculata*, through diligent and extensive study, has been found to have the following structure:



This product is a light yellow powder, slightly soluble in water and is relatively insoluble in alcohol, acetone, ether or benzene.

The new 2-amino-4-hydroxy-6-substituted pteridine of the present invention was obtained by adsorbing human urine on activated charcoal. The desired product was eluted from the charcoal with an aqueous alkaline or alcoholic alkaline solvent. The eluate was then diluted with a water immiscible alcohol. The water soluble impurities were separated from the active material by countercurrent distribution. Further impurities were removed by partition chromatography between water and an alcohol such as butanol or propanol. A mixture of the alcohol and a further solvent such as ethyl acetate can also be used. The partition chromatography process was repeated until the major portion of the impurities were removed. The desired product was then adsorbed chromatographically on magnesium silicate (Magnesol) and eluted with aqueous ammonia. The active product was then extracted into dilute mineral acid and solid impurities removed by filtration. The acid solution was then made alkaline and further solid impurities removed. The compound of the present invention was then obtained by neutralization with acid. Recrystallization from water removes any salt that may be present.

The new substituted pteridine was identified as having the 2-amino-4-hydroxy-6-dihydroxypropyl pterin structure based on the following experimental evidence and that

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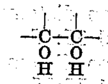
described at the end of the examples. The ultra violet and infrared absorption spectra is substantially identical with that of well known 2-amino-4-hydroxypteridines. The acid periodate oxidation product of the pteridine produces an ultra violet absorption essentially identical with 2-amino-4-hydroxy-pteridine-6-carboxylic aldehyde. The permanganate oxidation product of the pteridine had an ultra violet absorption curve essentially identical with that of 2-amino-4-hydroxypteridine-6-carboxylic acid. Permanganate and periodate oxidation products therefore indicated only one other substituent on the pteridine nucleus other than those in the 2 and 4-positions. The infrared absorption curve indicated the presence of alkyl hydroxyl groups and a methyl group. The periodate oxidation was indicative of the group



The absence of a primary alcohol was noted since if this had been present, it would have oxidized to formaldehyde in the periodate oxidation, and no formaldehyde was detected. Therefore, the side chain must have been the alpha-beta-dihydroxypropyl radical. The side chain is indicated as being in the 6-position when the permanganate oxidation product of the pteridine had an ultra violet absorption curve essentially identical with 2-amino-4-hydroxypteridine-6-carboxylic acid. The 2-amino-4-hydroxy-6-dihydroxypropyl pteridine decomposed on heating at a temperature within the range of 250°–280° C. Decomposition on heating without a definite melting point is characteristic of 2-amino-4-hydroxy-6-substituted pteridines. Analysis of the 2-amino-4-hydroxy-6-dihydroxypropyl pteridine for carbon, hydrogen and nitrogen agreed closely with the theoretical values for 2-amino-4-hydroxy-6-dihydroxypropyl pteridine. The distribution coefficients between aqueous buffers and n-butanol were as follows:

Aqueous buffer:	Distribution coefficient
pH 1.- N HCl	0.042
pH 3 0.05 M citrate	0.24
pH 5 0.05 M acetate	0.25
pH 7 0.05 M phosphate	0.24
pH 9 0.05 M borate	0.036

The following examples illustrate the method for obtaining the biologically active product of the present invention.

Example 1

A sample of 175 liters of human male urine adjusted to pH 5 was stirred at 25° C. for one-half hour with 1.75 kilograms of activated charcoal (Norite A), 1.75 kilograms of diatomaceous earth (Super-Cel) was added and the solid separated by means of a centrifuge. The solid was made into a slurry with 15 liters of an eluting mixture of alcohol, water, concentrated ammonium hydroxide (50:25:25). The slurry was poured into a 10 inch diameter glass column set on a perforated steel plate to which vacuum could be applied. The solid was eluted by filtration with the eluting mixture until a total of 80 liters of liquid was collected. The solid was discarded and the liquid was reduced in the still to about 2 liters.

The reduced eluate was made up to 3 liters of 0.05 M phosphate pH 5 and saturated with 3 liters of n-butanol. This was the starting material for a 40 tube 60 transfer-one liter per phase solvent countercurrent distribution between 0.05 M phosphate n-butanol. The tubes (6–12) containing the desired product were combined and reduced in the still to 250 ml. and and combined with another batch equivalent to 175 liters of urine purified by the same procedure to the same stage.